spectroscopic analysis of the effects on membrane structure and dynamics of two very different AMPs: the cationic PMAP-23, which creates pores according to the ''carpet'' model, and alamethicin, which forms ''barrel-stave'' channels. By using fluorescence anisotropy measurements on liposomes comprising probes localized at different depths in the bilayer, we measured peptide effects on membrane fluidity and order. Laurdan spectral shifts provided indications about water penetration in the bilayer. In the case of PMAP-23, it was possible to focus specifically on the lipids surrounding the peptide by following the membrane-probe fluorescence due to FRET from the peptide Trp residues. Finally, peptide-induced perturbation of lateral mobility and domain formation were determined by several methods. All experiments were compared with liposome-leakage measurements: while for PMAP-23 all membrane-perturbing effects are correlated with the vesicle leakage process, alamethicin does not significantly influence membrane dynamics at the concentrations in which it forms pores. Surprisingly, in all cases the most significant peptide-induced effect is a reduction in membrane fluidity.

3096-Pos Board B251

Understanding the Induction and Stabilization of Transmembrane Pores by Peptides

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Antimicrobial peptides of the innate immune system represent our first line of defense against infection. They exhibit a broad range of microbicidal activity encompassing Gram-positive and -negative bacteria, mycobacteria and spirochetes as well as some fungi and enveloped viruses. Common antimicrobial peptides act by forming transmembrane channels or pores.1,2 Despite these seemingly generic properties their activity toward certain cell types is often highly sequence dependent.3 This raises critical questions as to how these peptides discriminate between different cell types based on the membrane composition. Using molecular dynamics simulations, the structural properties of a range of pore forming peptides has been examined in solution and a variety of membrane environments. It is shown that pore forming peptides have a strong preference for regions of high membrane curvature and that the structure adopted is dependent on the degree of local curvature. The simulations further suggest that it is a correspondence between curvature of the peptide and the intrinsic curvature of the membrane that could account for the effect of variation in head group and tail length on antimicrobial activity.

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3097-Pos Board B252

Antimicrobial and Lytic Activities of Squalamine Analogs: Effect of Varied Cationic Groups at C3 and Stereochemistry at C7

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Squalamine (3β-N-1-{N-[3-(4-aminobutyl)]-1,3-diaminopropane}-7α,24R-dihydroxy-5α-cholestane-24-sulfate), an aminosterol from the dogfish shark (*Squalus acanthias*), is a potent antimicrobial compound believed to act through plasma membrane lysis. In this report, the antimicrobial activity of squalamine, several structurally related analogs, and bile acid based aminosterols are characterized using two different assays. The structurally similar analogs vary in the stereochemistry at the 3- and 7- substitutents, and also in the length and composition of the polyamine added at C-3. The bile acid analogs are generated by the addition of diaminopropane to C-3 of common bile acid methyl esters. The antimicrobial activity of the analogs was assessed against four bacterial strains. Membrane lytic activity was assayed using large unilamellar vesicles encapsulating a fluorescent calcein dye. Both vesicle lysis and antimicrobial activity of the aminosterols are shown to improve with more cationic groups on the polyamine substituent at C-3. Also, analogs with a 7α-OH substitutent are more effective than their 7β-OH analogs. The bile acid analogs were significantly less active than squalamine.The results suggest that the stereochemistry of the C7-OH group and the A/B ring junction is important in aminosterol activation at the membrane surface.

3098-Pos Board B253

Caught in the Act: Direct Measurement of Protein Translocation across Membranes using Droplet Interface Bilayers

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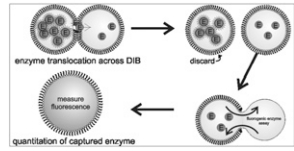
We introduce a new method for monitoring and quantitating the transport of materials across a model cell membrane. As a proof-of-concept, the cell-penetrating peptide, Pep-1, was used to carry horseradish peroxidase (HRP) across droplet-interface bilayers (DIBs). Two sub-microliter, lipid-encased aqueous droplets form a membrane at the contacting interface, through which enzyme-peptide complexes pass during transport. Following transport, the droplets are separated and the captured enzymes are assayed by a fluorogenic reaction. The DIB method recapitulates the findings of earlier studies involving Pep-1, including the dependence of protein transport on voltage and membrane charge, while also contributing new insights. Specifically, we found that leaflet charge symmetry may play a role in Pep-1-mediated protein translocation. We anticipate that the DIB method may be useful for a variety of transport-based studies, in particular those which must make use of tiny quantities of purified species.



3099-Pos Board B254

Balance between Additional Van Der Waals and Electrostatic Components of 107-115 hLZ Synergistically Increase Peptide-Membrane Interactions Potentiating its Antimicrobial Activity

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Substitution of Ala 108 and Ala 111 in the 107-115 human lysozyme peptide results in a 20 fold increase in the anti-staphylococcal activity and decrease its hemolytic activity which becomes significant only at 10 fold its MIC. This analog displays an additional positive charge near the N-terminus (108) and an extra Trp residue at the center of the molecule (111), suggesting that this constellation improves its interaction with the bacterial membrane. In order to understand the role of this arrangement in the membrane interaction, studies with model lipids membranes were carried out. The interaction of both peptides, 107-115 hLz and the novel analog ([K108 W111]107-115 hLz) were evaluated on liposomes by monitoring the fluorescence changes of its Trp residues and on lipid monolayers by monitoring the variation of the surface pressure. Results from both techniques revealed significant increase of lipid affinity of [K108 W111]107-115 hLz, specially in the presence of negatively charged lipid as POPG, but also a significant interaction with zwiterionics lipids that suggest that not only the electrostatic force are involved in the affinity increase. The peptides locations into membranes were also studied by fluorescence quenching techniques without any significant difference between both peptides, suggesting that the enhanced in the antimicrobial activity might be ascribed to an increased affinity. All together the data suggest that the enhanced activity of peptide [K108 W111]107-115 hLz might be explained by a synergy effect between the increased electrostatic forces (substitution of A by K at position 108) as well as the increased hydrophobic interactions (substitution of A by W at position 111). The substitutions might stabilize the peptide in the membrane.

3100-Pos Board B255

Coarse-Grained Simulations of Antimicrobial Action of Melittin and Magainin-2 on Phospholipid Bilayers

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The mechanism by which the antimicrobial peptides (AMPs) disrupt the cell membranes is not well understood. *In vitro* experiments have shown that the way different AMPs permeabilize membranes could be substantially different. Thus magainin-2, an AMP found in the skin of *Xenopus laevis* is found to cause efflux of dye from vesicle in the *all-or-none* mode, while melittin, the AMP found in the honey bee venom, is known to cause a *graded* release of the dye. Here we report coarse-grained simulations of melittin and magainin-2 on phophoplipid bilayers using MARTINI force field with both standard and polarisable water models. Simulations with DPPC bilayers using standard water model showed difference in peptide assemblies within the bilayer interior. While magainin-2 peptides aggregate in large number (17 to 19) and form larger disordered toroidal pores through which water flows, melittin formed smaller pores that leak water occasionally. Melittin channels contained 4-6 peptides, some in U-conformation. These results are consistent with dye efflux